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Screening of oil palm (*Elaeis guineensis* Jacq.) varieties for resistance to *Curvularia* leaf spot disease

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Abstract

Leaf spot disease, particularly *Curvularia* leaf spot (CLS), can devastatingly damage both quality and quantity of oil palm seedlings in Thailand. Chemical fungicides have been intensively applied to control transmission of this disease. However, this has relatively low efficiency as the causative pathogen gains resistance to fungicides with selection pressure. An alternative or complementary cost-effective and environmentally friendly approach is necessary to find the resistant varieties for disease management. In this present study, a total of 122 lines of Dura female plant, 2 lines of Pisifera male plant, and 4 Tenera commercial varieties (A, B, C and SUP-PSU1) were screened by *Curvularia* inoculation using detached leaf method. Two weeks after inoculation, the results showed 13 Dura lines as highly resistant to CLS (0% disease incidence), whereas one line (129) and the commercial variety B were highly susceptible (100% disease incidence). Nine Tenera hybrid lines from 13 Dura highly resistant lines were selected to test for high resistance and subjected to *Curvularia* inoculation in greenhouse conditions, and compared with the susceptible and highly susceptible lines. Three Tenera hybrid lines (138, 187 and 203) showed high resistance to CLS significant difference from susceptible lines. These were the most highly resistant varieties to CLS and should be considered for breeding programs of oil palm stock among the cases tested.

Keywords: *Elaeis guineensis,* leaf spot, pathogenicity, resistant varieties.
Abbreviation: HR_highly resistant, HS_highly susceptible, LSD_least significant difference, MR_moderately resistant, MS_moderately susceptible, R_resistant, S_susceptible.

Introduction

Oil palm is an important economic crop cultivated especially in southern and some parts of northeastern and eastern Thailand. To increase oil palm crop yield, factors to consider are plantation area, weather, agronomic methods and choice of plant variety (Corley and Tinker, 2003). Leaf spot of oil palm seedlings is the most serious problems in the common high-yielding Tenera variety (Pornsuriya et al., 2013). *Curvularia* leaf spot (CLS) caused by *Curvularia oryzae* has been reported to cause severe problems to many commercial varieties of oil palm seedlings (Sunpapao et al., 2014). This disease usually infects two-month-old transplanted seedlings and the seedlings remain susceptible until they reach 12-month-old (Turner, 1981). CLS is currently destructive and widespread (Pornsuriya et al., 2013; Sunpapao et al., 2014). The highest recorded incidence of CLS on oil palm seedlings (61.01%) in Southern Thailand was reported by Kittimorakul et al. (2013). During the rainy season, CLS epidemic is most severe due to the favorable environment for conidial germination and the spread by raindrops and winds between neighboring nurseries. Disease symptoms initially appear as small yellow spots on the leaves, then turn to light brown and further to dark brown covering most of the leaves (Kittimorakul et al., 2013). The pathogen produces spores massively.

Effective ways to control CLS include mechanical control and the application of fungicides, such as antracol, captan, mancozeb or prochloraz (Kittimorakul et al., 2014). However, extensive use of fungicides leads to development of resistant fungal strains. Accumulation of chemicals poses risk to human health and is harmful to the environment. A further alternative to address this problem is to screen the oil palm varieties for CLS resistance. Host-plant resistance is an effective and preferable method to manage plant diseases, and reduces fungicide usage supporting sustainable agriculture (Thakur, 2007).

Screening programs have been conducted by detached leaf method for finding resistance against foliar diseases in several plant species, and for pathogenicity in seedlings

(Tedford et al., 1990; St. Amand and Wehner 1995). This method is not only used in pathogenicity testing but also for fungicide screening, determining pathogen races, and genetically controlled resistance (Dhingra and Sinclair, 1995). In every breeding program, screening of resistance germplasm and testing for resistance of the segregating generations F_1 are really important before development and dissemination of new varieties (Dias et al., 1996). However, resistance to CLS in oil palm has not been previously screened in any breeding program. Therefore, the objective of this study was to screen and select the CLS resistance varieties in oil palm seedlings and to provide source plants to a breeding program. The detached leaf method was applied in a preliminary test of Dura and Pisifera parent breeding stock to select resistant varieties. The selected varieties were subjected to pathogenicity tests in oil palm seedlings and compared with Tenera variety as benchmarking (control) against a baseline.

Results and Discussion

Preliminary screening for resistance to leaf spot disease by detached leaf method

In this study, we tested the host response to CLS across a total of 124 lines including 4 commercial varieties. The results show response differences between the lines (Table 1). Based on the detached leaf method, the Dura variety 14 line showed the highest resistance (HR) with disease score 0 and 0% disease incidence. A total of 49 lines of Dura variety and 2 lines of Pisifera variety were resistant (R) (mean score 0.44 and 9.07% disease incidence), followed by 29 moderately resistant (MR) (mean score 1.56 and 32.74% disease incidence) and 24 moderately susceptible (MS) (mean 2.25 score and 47.36% disease incidence). A total of 3 lines were susceptible (S) with disease score of 3.44 and 72.74% (disease incidence), whereas 1 line was highly susceptible (HS) to leaf spot disease with disease score of 4.75 and 100% (disease incidence). Among the commercial varieties, SUP-PSU1 and variety C were rated as resistant, followed by variety A, rated as moderately susceptible. Commercial variety B had the highest disease score 4.75 with 100% disease incidence and was rated highly susceptible (Table 1). As described above, the detached leaf method was used for preliminary screening to select varieties resistant to CLS, scoring from 0 to 4.5. The mean score was converted to disease incidence, which was further categorized to six host response levels: HR, R, MR, MS, S, and HS.

The CLS is usually being found on young oil palm leaves. In pathogenicity tests by the detached leaf method, young to middle-aged leaves should be used for accurate results because leaf susceptibility depends on age (Dhingra and Sinclair, 1995). In this experiment, the leaflets on first leaf of oil palm at 6-7 years old age were collected to test, similar to natural infection. The detached leaf method has been used to screen for virulent fungal disease to control perennial weeds in North America (Yang et al., 1991). In addition, this is the basic method to select resistant varieties of various plant species, such as resistance to leaf spot in spring onion, resistance to white *Sclerotinia* and stalk rot in sunflower, foliar blight in soybean, and anthracnose in eggplant (Li et al., 2014; Irani et al., 2011; Rahayu, 2014; Saha et al., 2010). Thus, the detached leaf method was an easy and efficient system for the preliminary screening of oil palm varieties that resistance to leaf spot disease. The preliminary results require further validation under more realistic breeding conditions.

Screening for leaf spot resistant varieties in greenhouse conditions

The pathogenicity test of seedlings was carried out in greenhouse condition. A total of 12 Tenera F_1 hybrid lines/ variety (Dura × Pisifera) was tested and resistant and susceptible Dura female plant were screened (9 resistant, 2 susceptible and 1 highly susceptible) (Table 2). Line 187 (Fig. 2b) was highly resistant with disease score of 0.25 and 5% disease incidence, in which the CLS symptom appeared minimally (12 days post inoculation), followed by lines 203 (Fig.2c), 138 (Fig. 2a), 183, 188, 202/6, 207, 172 and 177 (Table 2). The line 129 was highly susceptible to CLS with disease score of 4.55 and disease incidence of 91% (Fig. 2d). This was the first detectable disease symptoms at 3 days post-inoculation (Table 2). The heredity of leaf spot resistance in oil palm may be partly a maternal effect, as this is a qualitative character of oil palm.

The pathogenicity test of seedling will be useful for inheritance studies where there are large numbers of plants per generation and crosses (Zhang et al., 1997). This test has also been used when there are many breeding programs on oil palm disease resistance such as vascular wilt disease (caused by *Fusarium oxysporum* f. sp. *elaeidis*) in Africa and basal stem rot (caused by *Ganoderma* spp.) in Southeast Asia (Ntsefong et al., 2012; Durand-Gasselin et al., 2005). The pathogenicity test method is also being used at nursery stage for testing oil palm progenies resistance against other disease (Durand-Gasselin et al., 2005; Rees et al., 2007). Therefore, screening of oil palm parents carrying the resistance gene by detached leaf method and tested inheritance in oil palm Tenera hybrid by pathogenicity test of seedling may be necessary or at least useful for developing new Tenera hybrid resistance to CLS varieties.

Materials and methods

Preliminary screening for resistance to CLS using detached leaf method

Plant materials

Experiments were conducted at Department of Plant Science and Pest Management Biotechnology and Plant Physiology Laboratory, Faculty of Natural Resources. One hundred and twenty-four oil palm lines (122 lines of Dura variety female plant and 2 lines of Pisifera variety male plant) were obtained from Oil Palm Agronomical Research Center and tested along with 4 commercial varieties Tenera hybrid: (A, B, C, and SUP-PSU1 used as susceptible control). At 6-7 years old stage of plant, the first leaflets of each oil palm genotype were collected. Then, the leaf apex and base was cut out, and only 20 cm of leaf length was used. Afterwards, the surface was sterilized with 70% alcohol for 30 sec. For each variety, three leaves were placed on a plastic tray, and the

Table 1. Disease score and disease incidence (%) of selected oil palm varieties including commercial varieties, 2 weeks after inoculation with *Curvularia oryzae* NK1.

Variety line number	Disease score [x]	Disease incidence (%) [y]	Host response [z]	Variety line number	Disease score [x]	Disease incidence (%) [y]	Host response [z]
19[D]	1.00	21.00	MR	133	1.67	35.11	MR
23	2.00	42.11	MS	134	2.08	43.84	MS
27	0.75	15.84	R	136	1.92	40.37	MR
28	0.58	12.26	R	138	0	0	HR
29	0.17	3.47	R	139	1.33	28.09	MR
31	0	0	HR	141	2	42.11	MS
32	0.67	14	R	142	0.08	1.74	R
33	0.17	3.47	R	145	2	42.11	MS
35	1.25	26.32	MR	146	2.08	43.84	MS
36	0.42	8.79	R	147	2.58	54.37	MS
39	0.08	1.74	R	148	2.34	49.16	MS
41	0.25	5.21	R	149	2	42.11	MS
42	0.58	12.26	R	151	2.50	52.58	MS
43	0.92	19.32	R	155	3.58	75.37	S
44	0.58	12.26	R	157	2.00	42.11	MS
46	0.50	10.53	R	159	0.75	15.79	R
47	0.75	15.84	R	163	1.08	22.79	MR
49	0.75	15.84	R	171	1	21.05	MR
50	1.17	24.53	MR	172	0	0	HR
51	0.59	12.32	R	175	2.67	56.16	MS
52	0	0	HR	177	0	0	HR
54	0.42	8.74	R	178	2	42.11	MS
56	1.33	28.05	MR	183	0	0	HR
57	2.00	42.11	MS	184	0.25	5.21	R
58	0.08	1.74	R	185	1.59	33.37	MR
59	0.58	12.26	R	186	1.84	38.63	MR
67	0	0	HR	187	0	0	HR
70	1.33	28.05	MR	188	0	0	HR
71	1.75	36.84	MR	189	0	0	HR
73	0.4	8.74	R	196	2.75	57.89	MS
74	0.58	12.26	R	198	1.67	35.16	MR
75	1.25	26.32	MR	201	2	42.11	MS
76	0.50	10.47	R	202	0.25	5.26	R
80	0.42	8.79	R	202/6	0	0	HR
81	2.5	52.63	MS	203	0	0	HR
82	1.84	38.63	MR	205	0.25	5.21	R
83	2.17	45.58	MS	206	0.75	15.79	R
84	2.25	47.32	MS	207	0	0	HR
85	1.92	40.37	MR	212	0.08	1.74	R
86	2.08	43.84	MS	213	0.08	1.74	R
90	1.84	38.63	MR	214	0.08	1.74	R
94	1.75	36.89	MR	215	0.08	1.74	R
96	0.92	19.32	R	221	0	0	HR
102	0.75	15.74	R	227	0.50	10.42	R
104	0.33	6.96	R	228	1.67	35.11	MR
105	1.08	22.79	MR	229	0.5	12.32	R
106	2.42	50.84	MS	230	0.25	5.26	R
107	1.75	36.84	MR	232	0.42	8.79	R
109	1.92	40.37	MR	235	0.33	6.95	R
110	1.58	33.32	MR	237	0.25	5.21	R
111	1.58	33.32	MR	239	0.17	3.47	R
112	2.17	45.58	MS	240	0.25	5.26	R
113	0.92	19.32	R	244	0.92	19.26	R
116	1.92	40.37	MR	246	0.08	1.74	R
117	3.00	63.16	S	248	0.25	5.26	R
118	3.75	78.95	S	255	0.17	3.53	R
119	1.75	36.89	MR	277[P]	0.92	19.32	R
123	2.84	59.68	MS	278	0.58	12.26	R
124	1.92	40.37	MR	Com. A	2.75	57.89	MS
127	2.33	49.11	MS	Com. B	4.75	100	HS
128	1.42	29.84	MR	Com. C	1.92	40.37	MR
129	4.75	100	HS	SUP-PSU1	1.18	43.79	MR
130	2.25	47.37	MS				
131	2	42.11	MS	LSD (0.05)	1.13	23.83	

[D]Dura variety female plant[P] Pisifera variety male plant [x]Using standard disease score from the detached leaves method . [y]Apparent fraction of leaf area infected with pathogen (%). [y]Using standard disease score from the detached leaves method . [z]Categorized host response to pathogen infection: 0% = highly resistant (HR), 1-20% = resistant (R), 21-40% = moderately resistant (MR), 41-60% = moderately susceptible (MS), 61-80% = susceptible (S) and 81-100% = highly susceptible (HS).*LSD =least significant difference.

Table 2. Delay to symptom appearance, disease score and disease incidence for 10 highly resistant varieties and 3 susceptible varieties at 20 days of post-inoculation.

Variety line number	Symptom delay after inoculation (days)	Disease score	Disease incidence (%)
117	3	3.10^{b}	62.00^{b}
129	3	4.55^{a}	91.00^{a}
138	10	0.40^{cde}	8.00^{cde}
155	3	3.70^{b}	74.00^{b}
172	9	0.95^{cd}	19.00^{cd}
177	5	1.05^{c}	21.00^{c}
183	7	0.90^{cde}	18.00^{cde}
187	12	0.25^{e}	5.00^{e}
188	10	0.65^{cde}	13.00^{cde}
202/6	7	0.60^{cde}	12.00^{cde}
203	10	0.35^{de}	7.00^{de}
207	7	0.75^{cde}	15.00^{cde}

Value are the mean, disease severity (%) caused by *Curvularia oryzae* NK1 (P<0.05), the value in the column followed by the same letter are not significantly different according to LSD test.

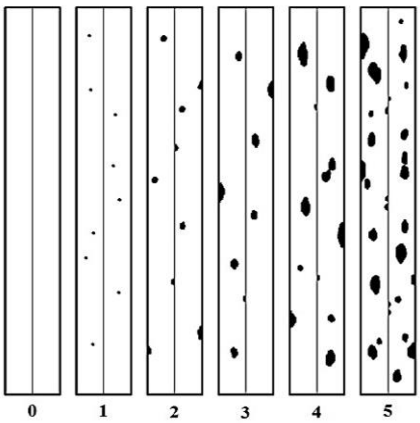

Fig 1. Standard disease severity scores: 0 = no disease symptoms; 1 = some pin-point brown spots on the leaf without any rotten tissue; 2 = less than 10 spots, 1–2 mm in size; 3 = less than 10 spots, 3–4 mm in size; 4 = less than 10 spots, ≥ 5 mm in size; 5 = more than 20 spots, ≥ 5 mm in size.

Fig 2. Disease progress on Tenera hybrid variety seedlings at 3 months age, in seedling trays at 20 days post inoculation. The lines are 138 (a), 187 (b), 203(c), and 129 (d).

experiment followed a completely randomized design (CRD) with four replicates (4 boxes). The pathogen virulent strain *C. oryzae* NK1 (Kittimorakul et al., 2014) was used for inoculation. The pathogen was transferred from stock onto corn meal agar (CMA) for sporulation and incubated at 25 °C for 20 days. Spore suspensions were prepared with sterilized distilled water and adjusted to 1×10^{6} conidia/ml. For the actual inoculation, 1.5 ml of spore suspension (1×10^{6} conidia/ml) was applied onto each oil palm leaf, followed by incubation in a moist chamber for 2 weeks (Kittimorakul et al., 2014). A box sprayed with sterilized distilled water served as the control. Disease assessment was scored with: 0 = no disease symptoms; 1 = some pin-point brown spots on the leaf without any rotten tissue; 2 = less than 10 spots, 1–2 mm in size; 3 = less than 10 spots, 3–4 mm in size; 4 = less than 10 spots, ≥ 5 mm in size; 5 = more than 20 spots, ≥ 5 mm in size (Fig.1).

The disease incidence of each line number was calculated as follows:

$$\% \text{ Disease incidence} = \frac{\text{Mean disease score of each line number}}{\text{Highest disease score of commercial variety}} \times 100$$

The disease incidence was used to evaluate the host response, to separate the oil palm varieties by different resistance level. Based on disease incidence, the host responses were assigned with one of the following six levels: 0% = highly resistant (HR), 1–20% = resistant (R), 21–40% = moderately resistant (MR), 41–60% = moderately susceptible (MS), 61–80% = susceptible (S) and 81–100% = highly susceptible (HS).

Response of oil palm varieties resistance to CLS in greenhouse conditions

Tenera F_1 hybrid varieties (Dura × Pisifera) were selected from resistant and susceptible Dura female plant and tested to compare CLS resistance in oil palm seedlings and to confirm the heredity of leaf spot disease resistance. In each line number, twenty oil palm seedlings at 3-4 months old stage were tested in a completely randomized design (CRD) with twenty replicates. The twenty seedlings were inoculated by spraying with conidial suspension of *C. oryzae* NK1 at 50 ml per tray (1×10^6 conidia/ml). The inoculated seedlings were subsequently covered with a plastic bag to maintain humidity and incubated for 48 h. The trays were then transferred into and kept in the greenhouse, with temperature varying in range of 25 - 30 °C. Days that CLS symptom appeared of each line number were recorded to compare symptom delay after inoculation between resistance and susceptible line. Twenty days after inoculation, the disease severity was scored for one of six severity levels: 0 = no lesions; 1 = lesions 0–20% of leaf area; 2 = lesions 21–40% of leaf area; 3 = lesions 41–60% of leaf area; 4 = lesions 61–80% of leaf area, coalesced to 1–2 cm in size; 5 = 75% of leaf area covered by lesions, leaf rotten. The mean disease score of each line was converted to disease incidence as described previously.

Statistical analysis

The disease incidence and the disease severity for each variety were averaged across the samples in each box, and the statistical analyses were conducted on the box means. The data were subjected to analysis of variance (ANOVA) using the generalized linear model (GLM) procedure in SPSS software (Version 16.0). For mean comparisons of the varieties to the baseline commercial variety, least significant differences (LSD) were evaluated where $P<0.05$ was considered significant.

Conclusion

The results demonstrate that lines 138, 187 and 203 were the most resistant to CLS according to both detached leaf method and pathogenicity test with seedlings. These lines will be shared with private nurseries and may benefit the oil palm breeding programs in Thailand.

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References

Corley RHV, Tinker PB (2003) The oil palm. 4th edn. Blackwell, London

Dhingra OD, Sinclair JB (1995) Inoculation of detached plant parts. In: Basic plant pathology method, 2nd edn. CRC Press, Florida, pp. 196-198

Dias RdCS, Queiroz MAd, Menezes M, De Queiroz MA (1996) Identification of sources of resistance to *Didymella bryoniae*. Hort Bras. 14:15-17.

Durand-Gasselin T, Asmady H, Flori A, Jacquemard JC, Hayun Z, Breton F, de Franqueville H (2005) Possible sources of genetic resistance in oil palm (*Elaeis guineensis* Jacq.) to basal stem rot caused by *Ganoderma boninense* - prospects for future breeding. Mycopathologia. 159:93-100.

Irani H, Heydari A, Javan-nikkhan M, Ibrahimov AS (2011) Pathogenicity variation and mycelial compatibility groups in *Sclerotinia sclerotiorum*. J Plant Prot Res. 51:329-336.

Kittimorakul J, Pornsuriya C, Sunpapao A, Petcharat V (2013) Survey and incidence of leaf blight and leaf spot disease of oil palm seedling in Southern Thailand. Plant Pathol J. 12:149-153.

Kittimorakul J, Petcharat V, Chuenchitt S (2014) Chemical and biological control of *Curvularia oryzae* leaf spot of oil palm. Songklanakarin J Pl Sci. 1:39-47.

Li MJ, Paul NC, Lee HB, Yu SH (2014) Characterization and pathogenicity of *Alternaria vanuatuensis*, a new record from Allium plants in Korea and China. Mycobiology. 42:412-415.

Ntsefong GN, Ngando G, Paul K, Martin BJ, Emmanuel YB, Hermine N, Gervais BE, Galdima M, Bienvenu A (2012) Control approaches against vascular wilt disease of *Elaeis guineensis* Jacq. caused by *Fusarium oxysporum* f. sp. *elaeidis*. J Biol Life Sci. 3:160-173.

Pornsuriya C, Sunpapao A, Srihanant N, Worapattamasri K, Kittimorakul J, Phithakkit S, Petcharat V (2013) A survey of disease and disorders in oil palm of Southern Thailand. Plant Pathol J. 12:169-175.

Rahayu M (2014) Identification and pathogenicity of pathogen responsible for aerial blight disease of soybean. J Exper Bio Agri Sci. 2:279-285.

Rees RW, Flood J, Hasan Y, Cooper RM (2007) Effects of inoculum potential, shading and soil temperature on root infection of oil palm seedlings by the basal stem rot pathogen *Ganoderma boninense*. Plant Pathol. 56:862-870.

Saha A, Isha M, Dasgupta S, Saha D (2010) Pathogenicity of *Colletotrichum gloeosporioides* (Penz.) Sacc. causal agent of anthracnose in different varieties of eggplant (*Solanum melongena* L.) determined by levels of cross-reactive antigens shared by host and pathogen. Arch Phytopath Plant Protec. 43:1781-1795.

St. Amand PC, Wehner TC (1995) Greenhouse, detached-leaf, and field testing methods to determine cucumber resistance to gummy stem blight. J American Soc Hort Sci. 120:673-680.

Sunpapao A, Kittimorakul J, Pornsuriya C (2014) Identification of leaf spot disease on oil palm seedling in nurseries of Thailand. Phytoparasitica. 42:529-533

Tedford EC, Miller TL, Nielsen MT (1990) A detached-leaves technique for detecting *Phytophthora parasitica* var. *nicotianae* in tobacco. Plant Dis. 74:313-316

Thakur RP (2007) Host plant resistance to diseases: potential and limitations. Indian J Plant Prot. 35:17-21

Turner PD (1981) Oil palm diseases and disorders. Oxford University Press, London.

Yang SM, Dowler WM, Johnson DR (1991) Comparison of method for selecting fungi pathogenic to leafy spurge. Plant Dis. 75:1201-1203

Zhang YP, Kyle M, Anagnostou K, Zitter TA (1997) Screening melon (*Cucumis melo*) for resistance to gummy stem blight in the greenhouse and field. HortScience. 32:117-121